EFFECT OF CERTAIN BACTERIA ON THE OCCURRENCE OF ENDOMIXIS IN PARAMECIUM AURELIA[1]

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It was at first believed that endomixis occurs in *Paramecium aurelia* at rather regular intervals, but detailed observations have shown that there is much variation in the length of the interendomictic period. Different stocks show differences in the average interval between endomixes: thus Sonneborn (1937) showed that, under identical conditions, in the stock *R* the mean interval between endomixes was 18.3 days (66.3 fissions), while in Woodruff's long-lived stock (*IV*) the mean interval was 46.3 days (110.4 fissions). Environmental conditions have been shown to alter the interendomictic period. Woodruff (1917) showed that sudden alterations in the cultural conditions bring on endomixis earlier than it would otherwise occur. Jollos (1916), by various changes in temperature and bacterial content of the culture medium, caused great changes in the length of the interendomictic period, from a minimum of 3 days to a period sufficiently great for 168 fissions to occur. Young (1917) increased the frequency of endomixis in *Paramecium aurelia* by alterations in the age of the culture medium and by changes in temperature. In *Paramecium caudatum,* Chejfec (1930) showed that the percentage of individuals undergoing endomixis in mass cultures was greater in a medium containing the bacillus coli than in hay infusion.

In the following study of *Paramecium aurelia* the frequency of endomixis and length of the interendomictic period were compared in culture media containing diverse bacteria, in different concentrations. Special attention was directed to the question whether it is primarily the unfavorable nature of conditions that brings on endomixis or increases its frequency.

A branch of Woodruff's long-lived stock of *Paramecium aurelia* was employed in the experiments. All lines of descent were derived from a single individual which underwent endomixis at room temperature during the first five-day period of the experiment (see Fig. 1). Thereafter all cultures were kept in a constant temperature box at about 25° to 26° C., except during the daily transfers.

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Five types of bacteria were employed in the different culture media, namely *Flavobacterium brunneum, Bacillus niger, B. cereus, B. prodigiosus,* and *B. coli.*

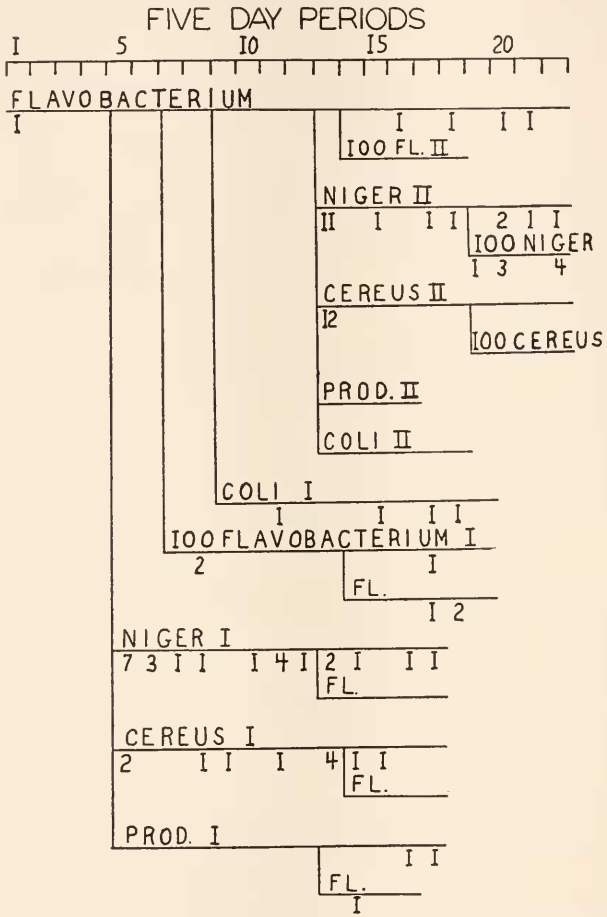

Fig. 1. Diagram of the course of the experiments. Each interval from left to right represents a five-day period. The horizontal lines show the number of five-day periods through which each culture (of 12 lines each) continued. The small numbers below the horizontal lines in certain periods give the number of lines (out of the 12) that underwent endomixis in that period. The heavy vertical lines show the source of the cultures; thus the culture "Niger I" was derived from the *Flavobacterium* culture at the end of its fourth five-day period: it continued through the seventeenth five-day period.

Flavobacterium, Flavobacterium brunneum; Niger, Bacillus niger; Cereus, B. cereus; Prod., B. prodigiosus; Coli, B. coli.

Designations preceded by 100 indicate cultures in which the concentration of bacteria was 100 times as great as normal; designations not preceded by 100 indicate the normal concentration. I and II indicate Groups I and II, mentioned in the text.

The standard culture medium was that commonly employed in this laboratory. It consists of infusion of desiccated lettuce leaves, into which is introduced the green alga *Stichococcus bacillaris,* in the proportion of three 2 mm. loops of the alga (from 20 to 22-day-old slants) to 20 cc. of the filtered fluid. To this is added a 1 mm. loop of *Flavobacterium brunneum* from a one-day-old agar slant. (For details see Sonneborn, 1936.) Since *Paramecium aurelia* flourishes well in this medium, it was employed as a norm or standard for comparing the results with the other bacteria.

For the other bacteria the medium was prepared in the same manner, with the substitution of equal amounts of the other bacteria for *Flavobacterium brunneum.* The medium is buffered to prevent the production of different pH by the diverse bacteria. In some of the experiments a nutritive medium was employed in which the quantity of bacteria was 100 times as great per unit volume as in the normal or standard medium. The purpose was to determine the results of preponderance of bacteria of a certain type, so that it was not considered essential to carry on the experiments under purely aseptic conditions; this would, for experiments of this nature, be practically impossible. Tests were made at intervals to determine whether there was marked contamination. No cross-contamination of the different bacterial types employed was observed in any case. There were three types of common laboratory contaminants; when these became at all abundant in the cultures (up to 30 per cent) such cultures were rejected.

For determining how far the effects of certain bacteria in certain concentrations are harmful or unfavorable, the following criteria were used. An unfavorable effect may be shown by (1) a slowing of the fission rate, (2) an increase in the proportion of deaths occurring, (3) incomplete division, resulting in the production of partially united chains of individuals. Accordingly, observations on these points were recorded for each different type of culture (see Table I). The occurrence of endomixis in the different media was detected by the daily staining of samples from each line.

The general plan of the experiments is shown in Fig. 1, on which is likewise indicated the number of lines in which endomixis occurred in particular five-day periods. In Table I are summarized the general results of culture in the different media. The following account of results is to be read in connection with the figure and table.

From a single individual that had just undergone endomixis, twelve lines were developed as quickly as possible, by cultivation in the normal *Flavobacterium* medium described above. This culture of 12 lines in *Flavobacterium* was maintained throughout the 22 five-day periods of

TABLE I

A summary of the effects of different bacterial media upon endomixis, the number of deaths and of chain-formation in Paramecium aurelia

Group	Total number of line-days	Mean fissions per line per five-day period	Total number of deaths	Number of deaths per 100 line-days	Total number of chains formed	Number of chains per 100 line-days	Total number of lines in endomixis	Number of lines in endomixis per 100 line-days
Flavobacterium brunneum								
Normal Concentration	1232	10.38	10	.81	2	.16	5	.41
100 Times Normal (Group I)	748	5.27	31	4.14	26	3.48	3	.40
Group I Returned to Normal	360	9.13	3	.83	3	.83	3	.83
100 Times Normal (Group II)	236	6.09	9	3.81	9	3.81	0	0
Bacillus niger								
Normal Concentration (Group I)	746	8.23	6	.80	0	0	23	3.08
Group I Returned to *Flavobacterium*	291	11.92	0	0	0	0	0	0
Normal Concentration (Group II)	590	8.91	9	1.53	0	0	18	3.05
100 Times Normal	240	9.82	3	1.25	0	0	8	3.33
Bacillus cereus								
Normal Concentration (Group I)	750	8.69	4	.53	0	0	11	1.47
Group I Returned to *Flavobacterium*	220	8.88	0	0	0	0	0	0
Normal Concentration (Group II)	297	8.93	3	1.01	0	0	12	4.04
100 Times Normal	225	4.61	7	3.11	7	3.11	0	0
Bacillus prodigiosus								
Normal Concentration (Group I)	772	8.88	7	.91	7	.91	2	.26
Group I Returned to *Flavobacterium*	207	8.48	0	0	2	.97	0	0
Normal Concentration (Group II)	197	9.42	4	2.03	4	2.03	1	.51
Bacillus coli								
Normal Concentration (Group I)	658	9.43	15	2.28	15	2.28	4	.61
Normal Concentration (Group II)	359	8.92	8	2.23	10	2.79	0	0

the experiments; it is represented by the upper horizontal line in Fig. 1. Cultures employed in the experiments with other media were branched off from this normal culture in particular five-day periods, as shown in Fig. 1. In each of the different media a group of 12 lines was kept in progress during a number of five-day periods, as indicated in the figure. The number of " line-days " given in column 2 of Table I is the number of lines of descent (usually 12, but with occasional irregularities), multiplied by the number of days during which the lines were in progress. In some cases two different groups of 12 were tested at different times in a particular medium; these are designated as I and II in the figure and table.

The main features of the diverse experimental cultures are as follows.

Flavobacterium brunneum: Normal or Standard Concentration

The 12 lines in this medium were kept in progress for 22 five-day periods, which covered the entire time of the experimental cultures. Total number of line-days, 1232. Mean fission rate, 10.38 per line per five-day period. Endomixis had occurred at the beginning of the culture; it occurred again in but four of the lines, in periods 16, 18, 20 and 21 (Fig. 1)—after intervals respectively of 75, 87, 97 and 103 days (168, 180, 212 and 224 generations). The remaining 8 lines did not undergo endomixis during the rest of the 110 days of the culture. The interendomictic periods are thus very long (as was before known to be the case in this stock). Thus, as the table shows, the total number of lines found to be in endomixis during this part of the experiment was but 5 (including that in the first generation), so that the number of endomictic lines per 100 line-days was but 0.41 (Table I).

In this medium the total number of deaths was but 10, or in the proportion of 0.81 per 100 line-days. Only 2 cases of united chains (incomplete division) occurred, thus but 0.16 per 100 line-days.

High concentrations of *Flavobacterium*.—At the beginning of the seventh 5-day period a group of 12 lines (Group I) was branched off and cultivated in the same medium, but with the concentration of bacteria 100 times as great. They were continued here till the end of the 19th period. As Table I shows, this decreased the fission rate to about one-half its former rate (5.27 in place of 10.38 per five-day period): multiplied the death rate by five and the proportion of incomplete fissions (chains) by about 22. Furthermore, the size of the individuals was notably decreased, and the contained crystals became larger and more numerous. Thus this high concentration must be considered distinctly unfavorable as compared with the normal. Seven days after the trans-

fer (in period 8, Fig. 1) two of the 12 lines underwent endomixis. A third line was in endomixis in the seventeenth five-day period: almost simultaneously (in periods 16 and 18) two lines were in endomixis in the normal concentration, so that no special significance can be attributed to this.

Thus the change to the unfavorable high concentration apparently induced endomixis in 2 of the 12 lines.

At the fourteenth period a new group of 12 lines was branched off from the normal concentration to the concentration 100 times as great (100 Fl II, Fig. 1) : it was continued through 4 periods. The unfavorable effects were shown about as before (Table I), but endomixis was not induced.

Restoration to normal concentration after culture at the high concentration:—In the case of Group I cultivated at the hundred-fold bacterial concentration, after seven five-day periods (at the 14th period of the experiment as a whole), a group of 12 lines was restored to the more favorable normal concentration. Fission rate was restored to normal, the proportion of deaths and of chains was greatly decreased (Table I). The normal size of the animals was restored after five days. Endomixis occurred in 3 of the lines in periods 17 and 18, but this cannot be attributed to the change to the normal concentration, since in the 100-fold concentration, as well as in the original culture in normal conditions, lines were in endomixis at practically the same times (in periods 16, 17, 18, Fig. 1).

Bacillus Niger

At the beginning of period 5, and again at the beginning of period 13, derivatives of the normal control group in *Flavobacterium* were washed and transferred to a medium containing *Bacillus niger* in place of *Flavobacterium* (see Fig. 1, " Niger I " and " Niger II "). The niger medium roughly corresponded in concentration to that of *Flavobacterium* (one 1-mm. loop to 20 cc. of culture fluid). In both cases there was a sharp lowering of the fission rate in the first period after transfer, but this was soon partially recovered, the average fission rate being 8.23 (Group I) and 8.91 (Group II) as compared with 10.38 in the entire *Flavobacterium* culture medium, so that these differences are hardly significant. As Table I shows, none of the other criteria indicate that *B. niger* is less favorable than *Flavobacterium*. Yet endomixis was at once induced in *B. niger*—in 7 lines in Group I, in 11 lines in Group II (Fig. 1). In all there were 23 lines in endomixis in Group I of *B. niger,* 18 in Group II—as compared with but 5 in the entire course of the *Flavobacterium* culture. The number of endomictic lines per 100

line-days was in *B. niger* a little over 3, as compared with 0.4 in *Flavobacterium* (Table I). In Group I, 9 lines underwent a second endomixis while in *B. niger,* and the interendomictic interval was short, ranging from 24 days (44 generations) to 48 days (79 generations), as compared with 75 to 103 days in *Flavobacterium.* Similar relations are found in Group II of *B. niger.*

It appears clear, therefore, that culture in *B. niger* greatly increases the frequency of endomixis, and that this is not a result of unfavorable conditions, for *B. niger* appears otherwise as favorable as *Flavobacterium.*

When derivatives from Group I in *B. niger* were restored to *Flavobacterium,* after 40 days in *B. niger,* there was after a few days a rise in fission rate. No endomixis occurred in those thus restored to the normal medium.

High Concentrations of B. niger

When individuals of Group II in *B. niger* were transferred to a concentration of *B. niger* 100 times as great as normal, there was no appreciable change in fission rate, mortality, or in number of imperfect divisions. Endomixis, however, was increased, 8 lines undergoing endomixis in the 4 periods of this culture (Fig. 1). This agrees with the fact previously noted, that the presence of *B. niger* in the culture medium increases the frequency of endomixis, and that this increase is not the result of the unfavorableness of *B. niger.*

Bacillus cereus

Two groups of 12 lines were transferred from *Flavobacterium* to a normal concentration of *B. cereus,* at periods 5 and 13 of the experiment (Fig. 1). Fission rate was very slightly decreased, and the animals were on the average a little smaller in size than in *Flavobacterium,* but mortality did not increase and there were no imperfect fissions, forming chains. Thus this bacillus can hardly be considered appreciably harmful. Yet, as in *B. niger,* endomixis was definitely increased in frequency. There were eleven endomixes in the 12 lines of Group I, 12 in those of Group II, while in the same periods the lines in *Flavobacterium* underwent but 4 endomixes. Seemingly *B. cereus* is less effective than *B. niger* in producing endomixis, since none of the lines in *B. cereus* underwent a second endomixis, while a number of those in *B. niger* did so, though the number of periods was the same for the two types of culture.

Restoration of lines of *B. cereus* culture to *Flavobacterium* did not result in the production of endomixis.

High Concentration of B. cereus

Derivatives of Group II in normal *B. cereus* were transferred at the nineteenth period to a concentration of *B. cereus* about 100 times as great (Fig. 1). This decreased the fission rate by about one-half, increased the mortality, and the formation of abnormal chains, and caused the production of large crystals in the cytoplasm. Conditions were clearly unfavorable, but endomixis was not induced.

Bacillus prodigiosus

At periods 5 and 13, two groups (I and II) were derived from the normal control cultures in *Flavobacterium,* and were cultivated in the normal concentration of *B. prodigiosus.* Fission was slightly lowered and the number of abnormal chains slightly increased (Table I); also a few individuals without macronuclei were observed. Thus the medium containing this organism must be considered slightly unfavorable. No increase in the frequency of endomixis was produced.

Bacillus coli

Groups I and II, derived in the usual way from the *Flavobacterium* cultures at the beginning of periods 9 and 13, showed little change in fission rate, but there was a considerable increase in the proportion of deaths, and of abnormal divisions (chains). Also numbers of individuals without macronuclei occurred. In Group I, four cases of typical endomixis occurred (Fig. 1). In addition, there occurred in most of the lines irregular fragmentation of the macronucleus. This began in both groups a few days after the transfer to *B. coli,* and continued for about 10 days. The fragments varied from day to day in size and number. They were scattered through the cytoplasm, or sometimes were in pockets of the macronucleus. The macronucleus was frequently present in two pieces; often it exhibited signs of activity, in the possession of projections of various sizes. Some of the stages closely resembled figures presented by Diller (1936, p. 37) for the process of hemixis in his types *B* and *C*. This fragmentation was in most cases not followed by the typical climax stage of endomixis, though it was in one of the lines. In other cases, after the disappearance of the fragments, the macronucleus remained normal. Such fragmentation occurred only in media containing *Bacillus coli.*

Summary

1. In the long-lived stock of Woodruff, cultivated in a standard medium containing a normal concentration of *Flavobacterium brunneum,*

endomixis occurred only at very long intervals, of 75 to 109 days, or more. Increase in the quantity of *Flavobacterium* to 100 times the normal concentration made the conditions very unfavorable, but hardly increased the incidence of endomixis.

2. Substitution of *Bacillus niger* for *Flavobacterium,* in the same proportions, brought on endomixis and increased its frequency, although it did not make the conditions unfavorable. Increasing by 100-fold the concentration of *B. niger* caused a further increase in the frequency of endomixis, but still without making the medium unfavorable.

3. *Bacillus cereus* was not appreciably unfavorable in its action on *Paramecium,* yet like *B. niger* it increased the frequency of endomixis. Its tendency to induce endomixis appears to be slightly less than that of *B. niger.* Increasing the concentration of *B. cereus* 100-fold made conditions very unfavorable, but did not increase the tendency to endomixis.

4. *Bacillus prodigiosus* is slightly unfavorable as compared with *Flavobacterium,* but it does not increase the frequency of endomixis.

5. *Bacillus coli* is unfavorable as compared with *Flavobacterium.* It produces a tendency to fragmentation of the macronucleus, usually not followed by complete endomixis.

6. Thus in these experiments there is no correlation between the unfavorable effect of certain bacteria and their tendency to produce endomixis. Certain species (*Bacillus niger* and *B. cereus*) are not unfavorable to *Paramecium aurelia* yet (in this stock at least) they much increase the frequency of endomixis. Certain other conditions that are unfavorable (particularly high concentrations of *Flavobacterium*) do not increase the frequency of endomixis.

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